ORIGINAL ARTICLE

COTTON-SWAB CRYOTHERAPY FOR ORAL LEUKOPLAKIA

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Abstract: *Background.* Oral leukoplakia (OL) is a common oral precancerous lesion. Cotton-swab cryotherapy (CSC) is commonly used for treating skin lesions but is rarely used for treating OL lesions.

Methods. Sixty OL lesions were treated by CSC once every 2 weeks until complete regression (CR) of the lesion had been achieved.

Results. CR was achieved in all 60 OL lesions after an average of 6.3 treatments with cryotherapy. The number of CSC treatments required to achieve CR was significantly fewer for OL lesions on oral mucosal sites other than the tongue, those <2 cm^2, those with epithelial dysplasia, and those with a surface keratin thickness of <55 μm. Multivariate analyses showed that only the location and area of the OL lesions were independent factors influencing the number of CSC treatments required to achieve CR.

Conclusion. For OL lesions with a mean surface area of ≤ 1.8 cm^2, CR can be achieved with fewer than 7 CSC treatments on average. CSC is a simple, safe, easy, conservative, and acceptable treatment modality for OL lesions. *Head Neck* **31:** 983–988, 2009

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Oral leukoplakia (OL) is a common precancerous lesion that may transform into squamous cell carcinoma. The malignant transformation rates of OL lesions are reported to be 1% to 7% for homogenous thick leukoplakia, 4% to 15% for granular or verruciform leukoplakia, and 18% to 47% for erythroleukoplakia.[1] The high malignant transformation rates of OL lesions highlight the importance of early detection and treatment of these lesions.

Although OL lesions can be eradicated by surgical excision, laser surgery, and photodynamic therapy,[2] cryotherapy is also an effective treatment modality for them.[3–11] Cryotherapy is a method that locally destroys lesional tissues by freezing in situ.[8] It is carried out with either an "open" or a "closed" system.[12] Open-system cryotherapy involves directly applying the cryogen to the lesion with a cotton swab[11] or using open spray.[12] Closed-system cryotherapy offers a

greater degree of control with a more-complex and -delicate apparatus.[11,12] The advantages of cryotherapy include bloodless treatment, a very low incidence of secondary infections, and a relative lack of scarring and pain.[11] Previous studies showed the utility of cryotherapy for treating various oral lesions[3–17] including OL lesions.[3–11]

The cotton-swab cryotherapy (CSC) technique, which is performed by directly applying liquid nitrogen to the lesion with a cotton swab, was first described by Toida et al.[13] It is a simple, easy method that requires no sophisticated skills or equipment.[13] Nevertheless, there are few studies using the CSC technique to treat oral lesions,[11,13–16] and only 1 study used the CSC technique to treat OL lesions.[11] In this study, we used the CSC technique to treat 60 OL lesions in 47 patients. Our main aim was to evaluate the efficacy of cryotherapy on OL lesions. Furthermore, relationships between the number of CSC treatments required to achieve complete regression (CR) and clinicopathological parameters of these 60 lesions were assessed by univariate and multivariate analyses.

PATIENTS AND METHODS

Patients and Oral Leukoplakia Lesions. Forty-seven patients (44 men and 3 women; mean age, 53 ± 13; range, 26–87 years) with a total of 60 OL lesions were recruited from the Department of Oral and Maxillofacial Surgery, National Taiwan University Hospital (NTUH) from July 2005 to July 2007. OL was defined as a white patch or plaque that could not be characterized clinically or pathologically as any other disease. A clinical diagnosis was confirmed by a histopathological examination of biopsy specimens taken from a characteristic part of the OL lesion at the patient's first visit. OL lesions were diagnosed as epithelial hyperplasia with either hyperkeratosis or parakeratosis when no dysplastic cells were found in the hyperplastic epithelium. Furthermore, OL lesions were diagnosed as exhibiting mild, moderate, or severe dysplasia when dysplastic cells were present in the basal one-third, basal two-thirds, or more than the basal two-thirds but not a complete layer of oral epithelium, respectively. The surface keratin thickness of each OL lesion was measured from hematoxylin and eosin–stained tissue sections with a built-in microscopic meter and is expressed as a mean of 5 measurements from 5 randomly selected areas.

All 44 male patients were both areca (betel nut) quid (AQ) chewers and smokers, but the 3 female patients had neither of these habits. All male patients stopped chewing AQ, but most of them continued to smoke a reduced number of cigarettes during the treatment period. Informed consent was obtained from each patient before the biopsy procedure and cryotherapy. This study was reviewed and approved by the Human Investigation Review Committee at NTUH.

Cotton-Swab Cryotherapy. We used a CSC technique to treat the OL lesions. Two kinds of cotton swab with diameters of 4 and 7 mm were used for the therapy depending on the size of the lesion. In brief, the lesion site was air-dried before treatment to prevent the cotton swab from sticking to the oral mucosa. The cotton swab was dipped into liquid nitrogen for at least 5 seconds and applied to the lesion with pressure for 20 seconds to form an ice ball and then allowed to thaw for another 20 seconds. Four consecutive freeze-thaw cycles were performed on the same area of the lesion. High-power suction was used to control saliva and vapor fog as well as for increased visibility. When the OL lesion was larger than 49 mm^2, each area of 49 mm^2 was treated separately until the entire lesion was completely treated. Most patients (n = 45) could tolerate the pain induced by the treatment. In 2 patients with severe pain, cryotherapy was carried out after topical application of 2% Xylocaine jelly. Choline salicylate gel was prescribed for each patient after cryotherapy to control the postcryotherapy pain if necessary. In 17 patients with moderate to severe postcryotherapy pain, an analgesic (acetaminophen, 500 mg/tablet, 1 tablet 3 or 4 times/day) was prescribed for patients after treatment. Clinical photographs were taken at each visit to evaluate the clinical outcome of cryotherapy. All 60 OL lesions were treated once every 2 weeks until CR of the lesion had been achieved. Patients were then followed up once a month for 3 months, once every 2 months for the next 4 months, and then once every 3 months thereafter. When the oral lesion recurred, it was treated with the same CSC protocol until CR of the lesion had been achieved.

Statistical Analysis. The relationship between the number of CSC treatments to achieve CR and each of the clinicopathological parameters was assessed for statistical significance by a chi-squared test (2 rows by 3 columns). Multivariate analyses using a Poisson regression model were used to assess which clinicopathological parameters were independent factors influencing the number of CSC treatments required to achieve CR. A p value of $<.05$ was considered statistically significant.

RESULTS

In total, 60 OL lesions from 47 patients were treated by the CSC technique. Of the 47 patients, 35 had 1, 11 had 2, and 1 had 3 OL lesions. The buccal mucosa ($n = 33$) was the most common site for OL lesions, followed by the tongue ($n = 11$), palate ($n = 6$), alveolar mucosa ($n = 4$), gingiva ($n = 4$), lips ($n = 1$), and floor of the mouth ($n = 1$). The surface area of the OL lesions varied from 0.1 to 6.5 cm^2 with a mean of 1.8 ± 1.5 cm^2. Histopathological examinations revealed that 19 OL lesions had epithelial hyperplasia with parakeratosis, 13 had epithelial hyperplasia with hyperkeratosis, 26 had mild dysplasia, and 2 had moderate dysplasia. The surface keratin thickness of the 60 OL lesions varied from 25 to 240 μm with a mean thickness of 55 ± 32 μm. The mean surface keratin thickness was significantly thicker in OL lesions without epithelial dysplasia (63 ± 39 μm, $p = 0.029$) or on the tongue (78 ± 63 μm, $p = .005$) than in OL lesions with epithelial dysplasia (45 ± 18 μm) or on oral mucosal sites other than the tongue (49 ± 16 μm), respectively.

Hyperemia and edema of the treated area occurred immediately after cryotherapy. Local swelling and bullous formation were evident during the following 2 to 3 days. Subsequently, superficial necrosis occurred, and the lesion was covered with a thin layer of a yellowish pseudomembrane. Epithelialization of each treated area was complete after 10 to 14 days, depending on the location and area of the lesion. The healing process of all lesions was uneventful, and most lesions healed within 2 weeks with no complications. Delayed healing of longer than 2 weeks was found in 3 relatively large lesions in 3 different patients. These 3 patients complained of severe pain after CSC treatment.

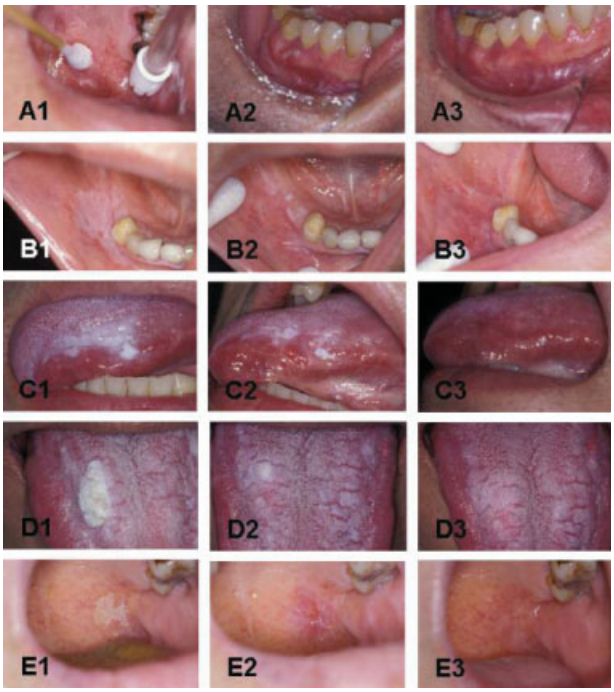

FIGURE 1. Clinical photographs of patients with oral leukoplakia (OL) before and after cotton-swab cryotherapy (CSC). (**A**) A cotton swab with liquid nitrogen was applied to the OL lesion on the left posterior palatal mucosa. High-power suction was used to control vapor fog and increase the visibility (**A1**). An OL lesion on the buccal gingiva between #45 and #46 before CSC (**A2**), and after 1 treatment with CSC showing complete regression (CR) (**A3**). (**B**) An OL lesion on the right buccal mucosa and right lower posterior edentulous alveolar mucosa before CSC (**B1**), after 3 treatments of CSC showing partial regression (PR) (**B2**), and after 4 treatments of CSC showing CR (**B3**). (**C**) An OL lesion on the left lateral border of the tongue before CSC (**C1**), after 6 treatments of CSC showing PR (**C2**), and after 12 treatments of CSC showing CR (**C3**). (**D**) An OL lesion on the right dorsal surface of the tongue before CSC (**D1**), after 12 treatments of CSC showing PR (**D2**), and after 17 treatments of CSC showing CR (**D3**). (**E**) An OL lesion on the left posterior palatal mucosa before CSC (**E1**), after 3 treatments with CSC showing PR (**E2**), and after 5 treatments of CSC showing CR (**E3**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

None of the lesions showed secondary infection after treatment.

All 60 OL lesions showed CR without scar formation after an average of 6.3 (range, 1–17) cryotherapy treatments (see Figure 1). The correlation between the number of CSC treatments required to achieve CR and each of the clinicopathological parameters of 60 OL lesions is shown in Table 1. We found that the number of CSC treatments needed to achieve CR for OL lesions on oral mucosal sites other than the tongue, those <2 cm^2, those with epithelial dysplasia, and those with a surface keratin

Table 1. Relationship between the number of cotton-swab cryotherapy (CSC) treatments required to achieve complete regression and each of the clinicopathological parameters of 60 oral leukoplakia lesions.

Clinicopathological parameter	Number of CSC treatments			Chi-square test *p* value
	1–6	7–12	13–17	
Age				.701
<50 y	16	7	3	
≥50 y	21	11	2	
Location				.003
Buccal mucosa	24	9	0	
Tongue	3	4	4	
Other oral mucosal sites	10	5	1	
Area				.024
<2.0 cm^2	30	9	2	
≥2.0 cm^2	7	9	3	
Epithelial dysplasia				.033
With	22	4	2	
Without	15	14	3	
Surface keratin thickness				.045
<55 μm	26	14	1	
≥55 μm	11	4	4	

thickness of <55 μm was significantly fewer than that for OL lesions on the tongue ($p = .003$), those ≥2 cm^2 ($p = .024$), those without epithelial dysplasia ($p = .033$), and those with a surface keratin thickness of ≥55 μm ($p = .045$), respectively (Table 1). Multivariate analyses using a Poisson regression model showed that only the location ($p = .000176$) and area ($p = .021280$) of the OL lesions were independent factors influencing the number of CSC treatments required to achieve CR (Table 2).

The follow-up period for the 60 OL lesions varied from 5 to 31 (mean, 17) months. During the follow-up period, 5 OL lesions (3 on the buccal mucosa, 1 on the tongue, and 1 on the gingiva) recurred 1 to 5 (mean, 2.1) months after cryotherapy. All 5 recurring OL lesions achieved CR again after an average of 3.4 (range, 2–5) treatments with cryotherapy.

DISCUSSION

Previous studies reported clinical outcomes of treating OL lesions with cryotherapy.[3–11] Sako et al[5] treated 60 patients with OL lesions using a special cryosurgical unit, and all OL lesions showed CR after 1 to 5 treatments. Chapin and Burkes[6] used cryotherapy with a gold cryoprobe to treat 4 patients with dysplastic and nondysplastic OL lesions and observed CR of all lesions after 1 or 2 treatments. Bekke and Baart[7] used cryotherapy with a probe to treat 35 OL lesions from 24 patients; all lesions showed CR after 1 to 4 treatments. Yeh[11] used the CSC technique to treat 102 oral lesions including 25 OL lesions. He demonstrated that small, superficial lesions showed CR after 1 treatment, but deep, large lesions needed 2 to 4 treatments to achieve CR.

In this study, we found that OL lesions of <2.0 cm^2 could be eradicated with a mean of 5.4 CSC treatments and with a mean of 8.1 treatments for those ≥2.0 cm^2. The greater number of CSC treatments needed to achieve CR in this study when compared with those reported in previous studies might have been due to the fact that we used a more conservative cryotherapy technique in which a constant low temperature was difficult to maintain in an open system. Moreover, we used high-power suction to control the vapor fog; in this situation, the liquid nitrogen may have easily evaporated from the cotton swab during the therapy. Therefore, destruction of the lesional tissue treated by our CSC technique might have been milder than those treated using special cryotherapy equipment that can maintain a lower, more constant temperature in lesional tissues during the entire treatment procedure.

Most animal tissues freeze at −2.2°C, and cell death occurs at a temperature of −20°C.[18] The mechanisms for cell destruction after cryotherapy are complex involving a combination of direct and indirect effects.[17] Direct effects consist of ice crystals that form in both extracellular and intracellular fluid, cellular dehydration, toxic intracellular electrolyte concentration, inhibition of enzymes, protein damage, thawing effects that cause the cell to vacuolate, swell, and rupture,[12,17] and thermal shock injury to cells.[17] Indirect effects include vascular changes

Table 2. Multivariate analyses of the number of cotton-swab cryotherapy treatments required to achieve complete regression and clinicopathological parameters of 60 oral leukoplakia lesions using the Poisson regression model.

Factor	Coefficient	*p* value
Intercept	1.67265	<2e-16
Location (tongue vs. other oral mucosal sites)	0.54983	.000176
Area (<2.0 vs. ≥2.0 cm^2)	0.26359	.021280
Epithelial dysplasia (with vs. without)	−0.08352	.482409
Surface keratin thickness (<55 vs. ≥55 μm)	0.13541	.228649

that lead to ischemic necrosis of the treated tissue and immunological responses that cause cell damage through a cytotoxic immune mechanism.[12,17]

Our study showed that OL lesions on the tongue needed more CSC treatments to achieve CR than those on other oral mucosal sites. The dorsal surface of the tongue is a specialized mucosa covered predominantly by a thick keratinized stratified squamous epithelium. This study demonstrated that OL lesions on the tongue had a significantly thicker surface keratin layer than those on other oral mucosal sites. Furthermore, the tongue is a highly vascular organ rich in capillary plexus in the long connective tissue papillae that are close to the mucosal surface.[19] We suggest that the thicker surface keratin layer on tongue lesions than on other oral mucosal lesions may act as a more effective barrier against the transmission of low temperatures into the underlying lesional epithelial cells. In addition, the rich blood supply in the lamina propria of the tongue may also contribute to a warming effect against tissue freezing and promote tissue recovery after cryotherapy.

This study found that smaller OL lesions needed significantly fewer CSC treatments to achieve CR than did larger lesions. Pogrel et al[20] showed that soft tissues at the center of the ice ball produced by liquid nitrogen ultimately undergo necrosis, while those at the margin of the ice ball usually do not reach sufficiently low temperatures to induce effective tissue necrosis. In this study, a larger OL lesion was divided into several small areas that were treated separately. Compared with the treatment of a larger lesion as a whole by special cryotherapy equipment, treatment of a larger lesion in sections using our CSC technique left multiple insufficiently treated margins and ultimately resulted in the need for more CSC treatments to achieve CR.

This study demonstrated a significantly fewer number of CSC treatments required to achieve CR for OL lesions with dysplasia than for those lesions without dysplasia. Histological examination revealed a thinner surface keratin layer on dysplastic OL lesions than on nondysplastic lesions. Thus, the need for fewer CSC treatments to achieve CR for dysplastic OL lesions may partly have been due to the thinner surface keratin layer on top of dysplastic lesions. Indeed, this study also showed that OL lesions with a surface keratin thickness of <55 μm needed significantly fewer CSC treatments to achieve CR than those with a surface keratin thickness of ≥ 55 μm. Moreover, dysplastic oral epithelium usually had wider intercellular spaces and contained more proliferating cells than did nondysplastic oral epithelium.[21] Wider intercellular spaces result in the formation of more extracellular ice crystals during cryotherapy, thus enhancing the tissue destructive effect caused by the therapy. In addition, proliferating epithelial cells are more sensitive to cryotherapy-induced tissue damage than are resting epithelial cells. These 2 reasons may also explain why dysplastic OL lesions required fewer CSC treatments to achieve CR than did nondysplastic OL lesions.

In this study, we successfully treated 60 OL lesions with the CSC technique. For OL lesions with the surface area of 0.1 to 6.5 (mean, 1.8) cm^2, CR of the lesion could be achieved with fewer than 7 CSC treatments on average. OL lesions on oral mucosal sites other than the tongue, those with a smaller surface area, those with dysplasia, and those with a thinner surface keratin layer required fewer CSC treatments to achieve CR than OL lesions on the tongue, those with a larger surface area, those without dysplasia, and those with a thicker surface keratin layer, respectively. We concluded that our CSC technique is a simple, safe, easy, conservative, and acceptable treatment modality for OL lesions.

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